Journal of Neuro-Oncology **64:** 3–11, 2003.

Overview

Brain tumor immunotherapy: an immunologist's perspective

Lois A. Lampson
CNS & Brain Tumor Immunology Laboratory, Department of Neurosurgery, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

Key words: brain neoplasms, glioma, immunotherapy, T-cell-mediated immunity, tumor antigens, tumor immunology, tumor vaccine

Summary

Key concepts in brain tumor immunotherapy are reviewed. "Immunotherapy" can refer to a fully-developed, tumor-specific immune response, or to its individual cellular or molecular mediators. The immune response is initiated most efficiently in organized lymphoid tissue. After initiation, antigen-specific T lymphocytes (T cells) survey the tissues – including the brain. If the T cells re-encounter their antigen at a tumor site, they can be triggered to carry out their effector functions. T cells can attack tumor in many ways, directly and indirectly, through cell-cell contact, secreted factors, and attraction and activation of other cells, endogenous or blood-borne. Recent work expands the list of candidate tumor antigens: they are not limited to cell surface proteins and need not be absolutely tumor-specific. Once identified, tumor antigens can be targeted immunologically, or in novel ways. The immune response is under complex regulatory control. Most current work aims to enhance initiation of the response (for example, with tumor vaccines), rather than enhancing the effector phase at the tumor site. The effector phase includes a rich, interactive set of cells and mediators; some that are not usually stressed are of particular interest against tumor in the brain. Within the brain, immune regulation varies from site to site, and local neurochemicals (such as substance P or glutamate) can contribute to local control. Given the complexity of a tumor, the brain, and the immune response, animal models are essential, but more emphasis should be given to their limitations and to step-by-step analysis, rather than animal "cures".

This special issue gives a snapshot of current work in brain tumor immunotherapy. The underlying logic, alternative interpretations, caveats and problems are as important as the technical findings. Often, the lead has been taken by neurosurgeons, rather than basic immunologists. To orient and welcome other non-immunologists, an immunologist's overview is offered here. The citation list includes all of the articles in the special issue, complemented by others where specific points are discussed more fully.

Tumor immunology: steps and principles

Defining immunotherapy

In current usage, 'immunotherapy' can refer to a fully developed immune response [1–3], or to exploitation of its component parts. The cells and molecular mediators that carry out the immune response also participate in other pathways, and they can be modified to perform new functions. Antibodies and lymphocytes can effect a classical immune response, or they can be exploited as vehicles to deliver novel agents to a tumor site. Mononuclear phagocytes can play multiple roles (Table 1). They can present antigen to initiate an immune response, and can act as final effectors in the immune attack initiated by antibodies or lymphocytes; they can also attack tumor independently. Still other examples are included in Table 1. Cytokines such *gamma interferon* (IFN-γ) or *tumor necrosis factor* (TNF-α) play multiple functions in the immune response, but also affect many other kinds of cells and functions [4–6]. Many examples of how the immune response and its components can be exploited against tumor are included in this special issue.

Table 1. Ways that mononuclear phagocytes within the brain may contribute to brain tumor attack

Role in tumor attack	Phagocyte acting as	Function
Help to initiate T cell response	Macrophage	Break down tumor antigen Carry ingested tumor antigen from brain to organized lymphoid tissue
Final attack of tumor	Antigen-presenting cell (APC)	Ingest and re-present tumor antigen to trigger T cell effector function
	Effector cell in immune response	Attracted and/or activated by lymphocytes to attack tumor
	Effector cell in 'innate' immunity	Direct attack of tumor, independent of immune response
	Vehicle	Blood-borne cells used to deliver agents to the tumor site
Identify tumor	Marker	Presence of activated phagocytes may help to mark micro-tumor sites [3]

This table lists some of the ways that mononuclear phagocytes can contribute to attack of tumor in the brain. Mononuclear phagocytes found in the brain include microglia, perivascular phagocytes, and blood-borne monocytes, all of which can take on the appearance of macrophages. Defining the roles that each populations plays, or may be made to play, is an important research goal. Further details are found in Refs [3–6].

A fully developed immune response

In the most traditional sense of the term, immunotherapy implies that an immune response is initiated against tumor antigen, and that immune effectors specifically attack the tumor where it grows. The approaches taken by Okada and colleagues [1] and by Liau and colleagues [2] provide complementary illustrations.

Okada et al. [1] report provocative clinical findings in a single human patient. The patient had been immunized with a vaccine that contained the patient's own tumor cells plus fibroblasts engineered to secrete the cytokine, interleukin (IL-4). The tumor was followed radiographically and T cells were analyzed at the vaccine site. In a complementary approach, Broder et al. [2] describe findings in a small animal model. Mice were immunized with a vaccine consisting of *dendritic cells* (DC) that had been engineered to express a defined tumor antigen. The anti-tumor activity of spleen cells from the immunized mice was analyzed *in vitro*. To show effects *in vivo*, Broder et al. [2] implanted tumor in the brains of vaccinated animals and controls, and measured survival. In each of these studies, the steps taken are based on current understanding of how the immune response is initiated and carried out (Figure 1). Key points of the underlying logic are reviewed below.

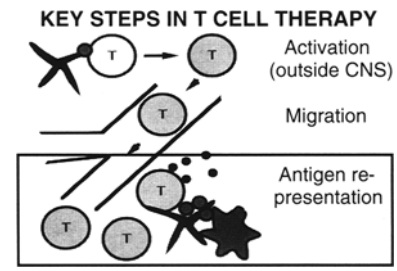

Figure 1. Key steps in T cell-mediated immunotherapy. T cell activation in lymphoid tissue (top), T cell migration (center), and antigen re-presentation to the T cell at the tumor site (boxed area) are shown. In peripheral lymphoid tissue, tumor antigen (small circle) is presented to a naive T cell (white T cell) (activation). The T cell undergoes clonal proliferation and the daughter cells (grey T cells) recirculate (migration). A T cell enters the cerebral vessels at random, and extravasates if appropriate adhesion molecules are expressed on the endothelial cells. If the T cell re-recognizes its antigen at the tumor site (antigen re-presentation), it can be triggered to carry out its effector functions. The tumor antigen can be re-presented by the tumor cell itself (dark grey shape) or by an APC that has ingested it (black star). Once the T cell has been triggered, it may attack tumor directly, or it may secrete cytokines or other factors (black discs) that attract or activate phagocytes or other cells to do so. Further details are found in Refs [3–6].

Classical steps in initiating and analyzing a tumor-specific immune response

(1) An immune response is initiated most efficiently in organized lymphoid tissue. Thus, it is appropriate to immunize peripherally (outside the brain), for example, intradermally [1] or subcutaneously [2]. From there, the antigen can be carried to draining lymph nodes or other organized lymphoid tissue.

It is also possible to immunize by injecting antigen into the brain. A conservative interpretation is that tumor antigen is carried from the brain to the cervical lymph nodes or other organized lymphoid tissue [7]. When intracerebral immunization appears preferable to other routes, the particular way the antigen is presented, or the particular balance of effector functions initiated in the cervical nodes may be factors [7]. Another factor is that

injecting antigen or other agents into the brain may, directly or indirectly, alter the environment for the effector phase of the response (as discussed below).

(2) In the course of successful immunization, naive T cells [8] are stimulated by their antigen to differentiate and begin clonal expansion; the differentiated daughter cells then enter the recirculating pool. Thus, it is appropriate to check for activated, tumor-specific T cells at the vaccine site [1], in the blood, or in the lymph nodes or spleen [2].

(3) The clonal progeny of activated T cells survey the tissues – including the brain. Although the physical blood–brain barrier prevents the passive entry of large proteins, it does not prevent the entry of metabolically active cells [4–6]. Thus, following peripheral immunization with tumor antigen, tumor-specific T cells can enter the brain.

(4) When migrant T cells re-encounter their antigen, they can be arrested and triggered to carry out their effector functions [3]. Thus, after immunization, activated T cells may be arrested at the vaccine site [1] or at the tumor site. Different subpopulations of T cells carry out different functions (as discussed below). Thus, it is of interest to characterize the subset composition of the accumulated T cells [1,9].

(5) It is important to distinguish between T cell accumulation and T cell function. The number of T cells observed at the tumor site may be poorly correlated to their functional impact. T cells can amplify their own functions by attracting and activating phagocytes and other cells. Once they have carried out their effector functions, T cells may leave the site or die. In cases where evidence of T cell attack is seen, but relatively few T cells are detected [2,10–12], both these factors may contribute.

Conversely, T cells can accumulate non-specifically in response to cytokines or other immune-enhancing agents, at sites of damage, inflammation, or an ongoing (unrelated) immune response; they can be stimulated to carry out regulatory functions rather than tumor attack; and their anti-tumor activity can be suppressed [6,8,9,13], as discussed in more detail below. Each of these factors may contribute when, despite evidence of T cell accumulation, tumor control is not achieved.

A further caveat is that T cell function is controlled locally. Evidence of T cell function outside the brain or *in vitro* does not necessarily imply successful tumor attack within the brain. Moreover, manipulations designed to stimulate T cell function can also stimulate other forms of tumor attack, for example, by phagocytes (Table 1) or *natural killer* (NK) cells [13]. To fully exploit observed efficacy, it is important to define which of the many potential effector mechanisms are most important at the tumor site.

A defining criterion for classical, antigen-specific immune activity is specificity. The traditional test is to compare the response against tumor that bears the immunizing antigen *versus* tumor that does not. The traditional way to establish a particular effector function is to block, deplete, or add that function, and compare the results to controls. In reading the emerging literature, it is important to ask about how well the underlying effector mechanisms are understood.

Tumor antigens: twists and turns

By definition, tumor immunotherapy depends on the tumor expressing a target antigen. New understanding and new tools are each contributing to a growing list of potential target antigens and ways of exploiting them [14–18].

Candidate tumor antigens

Conceptually, one important point is that tumor antigens are not limited to cell surface proteins. The reason is that T cells (which carry out the cell-mediated immune response) recognize processed antigen, not free proteins. The ability of an internal protein to serve as a brain tumor target antigen, previously shown for an artificial antigen [19], can now be exploited for *bona fide* brain tumor antigens.

Although some tumors can process and present their own antigens, this may be inefficient for brain tumor cells [3], as discussed further below. Fortunately, any protein can be ingested, processed, and presented by an *antigen-presenting cell* (APC). In the brain, potential APC include parenchymal microglia, perivascular phagocytes, and blood-borne monocytes; their relative contributions are a topic of much current interest.

Another important concept is that a tumor target antigen does not have to be absolutely tumor-specific. A normal protein may serve as a tumor marker if it is over-expressed in tumor cells as compared to other cells that would be found at the tumor site [14]. A normal protein may serve as a tumor target if the normal cells which express it can be protected from the therapy or if they are expendable [5].

Several antigens that may be of practical use, even though they are not absolutely tumor-specific, are explored in this special issue. Katoh et al. [14] have examined the distribution of the candidate antigen, survivin, paying particular attention to its expression in tumor cells *versus* tumor-infiltrating cells. Parsa and colleagues [15] explore the potential of a patient's own fibroblasts to serve as a source of practically useful antigen. The approach taken by Kruse and colleagues follows from the fact that the major histocompatibility antigens are expressed weakly, at best, on normal neural cells [20]. The paper by Paul et al. [16] continues their exploration of the potential of histocompatibility antigens to serve as practical brain tumor targets. Characteristic features of several other brain tumor antigens are described in the review by Rustamzadeh et al. [17].

How many antigens are needed?

As the list of defined tumor antigens grows, it is of interest to ask how complex a mixture is optimal for immunization [5]. A single defined peptide gives the greatest control over the kind of response that will be initiated and potential cross-reactions with normal tissue. On the other hand, immunizing with a mixture of antigens, besides permitting attack of multiple targets, also stimulates a greater variety of effector functions [6]. Both factors are desirable for attacking a heterogeneous, growing tumor. If a targeted antigen is not essential to tumor growth, antigen-negative variants can grow out, particularly under the selective pressure of immunotherapy. Tumor variants that are resistant to individual effector mechanisms may be selected as well.

Although a relatively simple immunogen may give better control over the initial immune response, the response can still change with time. Even if a single peptide is used to immunize, new antigens can be targeted as the response develops, a process now known as epitope spreading [21]. Indeed, using 'strong' antigens to help initiate an immune response is one classic strategy for stimulating a response that ultimately targets bona fide tumor antigens [5], a strategy that is still being used in new ways [22].

It is too soon to know how these different considerations will play out in practice, in human patients. In reading the current literature, it is helpful to be aware of the complexity of the antigen, and the authors' reasons for their choice. Among the following articles, a full range of possibilities is used to stimulate an anti-tumor response: a defined antigen [2], whole tumor cells or homogenates [1], and even immune-stimulating agents without an extraneous source of tumor antigen (other than the growing tumor itself) [22].

A developing immune response may target, not only additional tumor antigens, but also normal tissue, and this has caused great concern about the safety of immunotherapy. Fortunately, there is reason for optimism. In general terms, evolution of the response is a normal component of immunity. Not all cross-reactions with normal tissue are harmful, and some may be beneficial, contributing to homeostasis or immune control [6]. Growing basic understanding of immunity in general, and CNS autoimmunity in particular, makes it more likely that unwanted responses can be avoided or brought under control [6]. Balancing these factors against the grim short-term prognosis for many forms of CNS tumor, continued efforts toward immunotherapy seem well-justified.

Conventional and novel uses

As new tumor antigens are being defined, they are also being exploited in new ways. Fundamentally, a tumor antigen is a tumor-associated molecule that is abnormal in some way: it may be mutated, over-expressed, or expressed by inappropriate cells or in an inappropriate regulatory context. The review by Rustamzadeh et al. [17] offers a broad perspective on how such abnormal molecules can be targeted. Certainly, they can be attacked by conventional immune effectors (T cells or antibodies), or by effectors whose activity has been modified or amplified. Many examples of antibodies that have been truncated (which may enable them to cross the blood–brain barrier) or coupled to toxins (immunotoxins) are included in the review [17].

Alternatively, an abnormal molecule can be targeted by virtue of its particular characteristics, as Rustamzadeh et al. [17] also describe. For example, a tumor-associated receptor can be targeted by coupling its normal ligand to a toxin. One such toxin is the focus of the therapeutic approach being developed by Debinski and colleagues, as described in the article by Mintz et al. [18]. In that case, a glioma-associated cytokine receptor (for IL-13) is targeted by coupling its normal ligand, or a mutant variant, to a toxin (cytotoxin). Whether targeted immunologically or in other ways, tumor-associated abnormalities can also be exploited to image tumors, to classify them [25],

and, perhaps most important, to probe their underlying biology.

Activation, tolerance, and suppression

An individual may respond to a potential tumor antigen as 'foreign', initiating an immune response, or as 'self', displaying immune tolerance. The previous immunologic history and the way the antigen is presented are both factors. The distinction between self and foreign is not fixed [5]. Tolerance can be induced or broken and, as Prins et al. [8] point out, fresh T cells can be exported from the thymus. The potential importance of recent thymic emigrants (RTE) for brain tumor patients, and how RTE may be influenced by the tumor, are explored in the paper by Prins et al. [8].

The outcome of immune recognition is controlled throughout the response. As the normal response unfolds, feedback controls and regulatory cytokines help to limit and complete it. One way that tumors can escape immune attack is by distorting the normal controls, for example, secreting immunosuppressive cytokines. One well-studied example, TGF-β, is the focus of the paper by Withan et al. [13].

Yu and colleagues [9] bring out yet another way in which tumor cells may escape immune attack. One of the major pathways by which T cells can directly kill tumor requires interaction between Fas ligand (FasL) on a tumor-specific T cell and Fas on the antigen-bearing tumor cell. Recent work suggests several ways in which this pathway may be distorted. Fas or FasL may be lacking, they may be expressed on competing cell types, or soluble inhibitors may be present. T cells themselves express Fas, and so can themselves be targets of Fas/FasL interaction [6,9]. Further complicating the picture, Fas and FasL, like so many other molecules, can serve multiple functions, and not all functions are directly related to immune attack [6,9]. Implications of FasL expression on tumor-associated endothelial cells are explored in the paper by Yu et al. [9], where many aspects of Fas/FasL biology are introduced.

Although it is well-established that brain tumors, like other tumors, can be immunosuppressive, the suppression is not necessarily equal for all functions or at all tumor sites. Robust effects of IFN-γ in tumor-bearing brains, stressing micro-tumor sites, are described in the paper by Dutta et al. [3]. More broadly, it is useful to think in terms of a dynamic balance between cells and molecular mediators that favor tumor growth and those that contribute to tumor control. Provocatively, a given cell or mediator may contribute to each process, with the net effect often difficult to predict [26]. This is one reason that the selection and interpretation of *in vivo* models requires so much care, as discussed in the final section.

Enhancing the response: immuno-gene therapy and DC vaccines

Two of the main strategies that are now being explored to enhance the immune response are the introduction of immuno-active molecules (such as cytokines), often in the form of gene therapy (*immunogene therapy*) [1,22–24], and DC vaccines [2,27,28]. Several examples of each type are included within this special issue. Points that contribute to the underlying logic and interpretation are reviewed below.

Peripheral vaccines versus *changes to the tumor site*

It is important to distinguish between two aspects of the immune response, the initiation and effector phases. An immune response is initiated most efficiently in organized lymphoid tissue. The activated cells undergo clonal expansion, and the daughter cells survey the tissues (including the brain). The effector phase is triggered when the daughter cells re-recognize their antigen at the tumor site (Figure 1). Many immunotherapy studies have focused on the initiation phase – identifying tumor antigens, and stimulating more effective immune responses against them. Ways of enhancing the effector phase merit more attention.

Usually, but not always, tumor vaccines are delivered to the periphery (not the brain) (Figure 1), while attempts to enhance the effector phase involve delivering material to the tumor site. Peripheral vaccines and intracerebral injection of immune-enhancing material are both described in this special issue, as reviewed below.

Peripheral vaccines

Many laboratories are now working to develop tumor vaccines. One approach is to exploit DC, which can play multiple roles in initiating the immune response. They can ingest antigen, carry it to draining lymphoid tissue, and present it to antigen-specific lymphocytes. Several variants of DC-based tumor vaccines are described in this special issue. For their *in vivo* study,

Broder et al. [2] immunized with DC that had been engineered to express a defined tumor antigen. Working *in vitro*, Sloan and Parajuli [28] fused DC to tumor cells and compared this to other preparations. Many other examples are included in the broad overview of DC biology by Fecci et al. [27].

In parallel, there has been sustained interest in using cytokines, growth factors, or co-stimulatory molecules to enhance initiation of the anti-tumor response [5]. Strategies include immunizing with tumor cells that have been engineered to express such molecules, or immunizing with tumor cells or tumor antigen plus a separate source of the immune-stimulating molecule of interest [1].

Changes to the tumor site

Several papers describe the effect of introducing cytokines or other immune-stimulating factors to the tumor site [3,22–24]. A variety of delivery methods have been used, with the level, rate, and duration of delivery among the important considerations [22–24]. In the animal studies of Glick and colleagues [22], IL-2 secreting allogeneic fibroblasts were injected into the brain and, to test protection, tumor cells were later introduced into the same site. In the on-going phase I/II clinical trial being carried out by Rainov and colleagues, a liposome-encapsulated viral vector carrying the IL-12 gene is introduced by intra-tumoral infusion, as described by Ren et al. [23]. In the animal studies of Dutta et al. [3], a bolus injection INF-γ was made into the tumor-bearing brain.

When intracerebral injection of cytokine or other material is found to affect tumor growth, several pathways could contribute. Some of the possibilities are discussed below.

(1) Initiation of the immune response may be enhanced. For example, an injected cytokine may activate local phagocytes, and this in turn may enhance processing of the tumor antigen or its delivery to organized lymphoid tissue.
(2) A response may be initiated against the injected material and then evolve to target tumor antigens (epitope spreading, discussed above). This mechanism would be especially relevant when histo-incompatible fibroblasts have been injected, as discussed by Glick et al. [22].

    The use of histo-incompatible fibroblasts is also of interest for other reasons, as Glick et al. [22] point out. Among them, it raises the possibility that the fibroblasts to be injected do not have to be obtained from the tumor-bearing patient. Taking this approach a step further, Lesniak et al. [24] explore the use of xenogeneic cells and cytokines (from other species).
(3) Material that is injected into the tumor site may act, directly or indirectly, to enhance the effector phase of the response, for example by enhancing the accumulation or activation of T cells, NK cells, or phagocytes [3,13,22,23].

As a specific example, consider the multiple effects that injected cytokines may have on T cell-mediated attack. Immunization (vaccination) produces a pool of recirculating, tumor-specific T cells, as described above. To trigger tumor attack, the minimal requirements are that these T cells migrate to tumor sites, and that tumor antigen be re-presented to them (Figure 1). Injected cytokines can enhance this process at several steps [3,4,29–31]. For example, intracerebral injection of IFN-γ or TNF-α can safely increase extravasation of activated T cells and monocytes from the blood, presumably by increasing expression of relevant adhesion molecules on cerebral endothelial cells [4,29]. The same cytokines can also activate phagocytes, which can contribute to tumor attack in many ways (Table 1).

Cytokines such as TNF-α or IFN-γ can also kill tumor cells directly, but it has been difficult to deliver the necessary doses without unacceptable toxicity. Lower doses appear to be sufficient for the immune-enhancing effects described above (increasing T cell entry and the number of activated phagocytes). Paradoxically, these same cytokines may be secreted by effector cells in the course of tumor attack. However, in that case, general toxicity is avoided because the effector cell secretes the cytokine right at the tumor site [6].

The discussion above illustrates a few of the ways that immune attack might be affected by intracerebral cytokine injection. Other examples and perspectives are found in several articles in this special issue, especially those by Glick, Lesniak, Ren, Withan and their colleagues [13,22–24].

The effector phase: variety and heterogeneity

Effector populations, functions, and mediators

The great variety of immune effector mechanisms and molecular mediators should be stressed.

The most basic demarcation is between the cell-mediated immune response (carried out by T cells) and the antibody-mediated response, which have complementary advantages and limitations.

Antibodies can bind directly to tumor antigen; antigen processing is not required. However, binding of antibody is not, of itself, enough to kill a tumor cell. Common strategies have been to couple antibodies to radionuclides, with the aim of imaging tumor; or to radionuclides or toxins, with the aim of attacking it. A second limitation is that an antibody molecule is too large to cross the intact blood–brain barrier. The increasing sophistication with which antibody fragments can be exploited is well-illustrated in the review by Rustamzadeh et al. [17].

T cells can attack tumor in several ways. When T cells attack via mechanisms that require cell–cell contact, they are often referred to as *cytotoxic T lymphocytes* (CTL). Although most studies of cell-mediated tumor therapy have stressed the CTL response, it has important limitations. Direct T cell/tumor interaction requires that the tumor cell process and present its own antigen to the T cell. Many tumor cells, including many brain tumor cells, show at best weak expression of the major histocompatibility complex (MHC) proteins that are required for antigen presentation, and tumor cells can also be defective at other points in the antigen presentation pathway [32,33]. Fortunately, T cells can also recognize tumor indirectly. This occurs when phagocytes process and present tumor antigen they have ingested. This can trigger the T cells to secrete factors that kill adjacent tumor cells as bystanders, and factors that attract and activate other cells to do so (Figure 1).

T cells are subdivided into two major populations, marked by the cell surface proteins CD8 and CD4, respectively. Although, in fact, the functional potentials overlap, CD8+ T cells are considered most important for CTL attack, and CD4+ T cells for indirect attack. CD4+ T cells are further subdivided according to the patterns of cytokines they secrete. In analyses of the T cell subset composition *in vivo*, these functional distinctions naturally influence the interpretation. However, this is at best an indirect indication of the actual effector function, as discussed at the beginning of this review.

Although MHC proteins are required for all conventional antigen presentation, different sub-families, class I or class II, are required for presentation to CD8+ and CD4+ T cells, respectively. In studies of tumor therapy, CTL function is usually stressed. However, the flexibility of CD4+ T cells (which need not recognize tumor directly), and the characteristic pattern of MHC expression in the brain (with class II MHC on potential APC predominating [3,20]), suggest that CD4+ T cells may be of special value against tumor in the CNS [3–6].

Of course, T cell are not the only cellular effectors that can attack tumor. Natural killer cells and mononuclear phagocytes can also distinguish between tumor and normal cells. Although the molecular bases for their recognition of tumor are not fully understood, they are known to be different from those used by T cells. The potential value of NK cells has been stressed by Chambers and colleagues, as discussed in the paper by Withan et al. [13].

Local control

Most often, the immune response to tumor in the brain is thought of at the level of the whole organ. However, there is accumulating evidence of site-specific immune control [34]. This implies that the optimal regimen for immunotherapy may vary with the tumor site. For example, the brainstem is particularly responsive to the immune-activating cytokine IFN-γ, which may favor immunotherapy for brainstem glioma [5,34]. One contributing factor is that local neuropeptides (such as substance P) and neurotransmitters (such as glutamate) can influence local immune control [34].

Site-specific immune regulation may not be relevant for a large tumor mass, which presumably creates its own immune environment. However, it would be relevant to smaller foci of disseminated or residual tumor (micro-tumor). Although conventional therapies are increasingly successful against larger tumor masses, a well-established feature of glioma biology is that, after conventional therapy, infiltrative or disseminated micro-tumor remains. Similarly, as primary and metastatic tumor in other organs comes under better control, brain-metastasizing tumor, which would first appear as micro-metastases, is increasingly important as the cause of failure.

Actively motile T cells, already adapted to move through tissue and selectively attack abnormal targets, seem well-suited to attack micro-tumor, especially if the efficiency of T cell/tumor interactions can be increased. The local concentration of tumor-associated immunosuppressive factors would be less at micro-tumor sites than within a large tumor mass. Provocatively, T cell-mediated immunotherapy may

thus be best suited to the case in which it is most needed: to reach and attack primary or metastatic micro-tumor that is not readily imaged or accessed by other means [4–6].

A hard look at animal models

Given the complexity of tumor biology and immunology, it is not surprising that ideas must be developed through many small steps. A typical progression is to test a new concept or method *in vitro*, then in small animal models, first for tumor growing under the skin, then for tumor growing in the brain. *In vivo* studies are often broken down into still smaller steps, in which tumor control is expected to be progressively more difficult. Treatment given before tumor is implanted (protection/prophylaxis) [2,19,22], treatment given at the same time as tumor implantation [24], and treatment of animals with established tumor (therapy) [18]. In reading the emerging literature, it is important to keep in mind where any given study lies along the spectrum. The relationship between a tumor and its environment is dynamic. The extent of immunosuppression would be greater for an established tumor, and other variables would change as well.

The translation from successful animal studies to successful clinical trials has often failed. This does not invalidate the use of small animal models, but it does stimulate re-evaluation of how they are being used. Often, there is great pressure to design an animal study to show efficacy, especially 'cures'. Greater emphasis on step-by-step understanding, on seeking ways to stress the models, and on identifying caveats and weaknesses should be rewarding [35]. Great care is needed, not just in applying findings from experimental models to human patients, but at each intermediate step: from tumor-derived cell lines to primary tumors, from culture to animals, and from subcutaneous sites to the anatomic and pharmacologic complexity of the brain. Similar care is needed in modeling the appropriate biology for primary brain tumors *versus* blood-borne metastases from other sites [35].

Conclusion

The idea that the brain is 'immunologically privileged' is being discarded, and replaced with appreciation that, as for other organs, immune activity is under regulatory control [3–6]. The articles in this special issue illustrate the range of therapeutic approaches that are now being explored against CNS tumor, and the logic behind them. The overview above is offered to help non-immunologists appreciate and interpret the emerging literature and see how their own expertise might contribute to this rapidly developing field.

References

1. Okada H, Lieberman FS, Edington H, Witham T, Wargo MK, Cai Q, Elder E, Whiteside T, Schold SC Jr, Pollack IF: Autologous glioma cell vaccine admixed with IL-4 gene transfected fibroblasts in the treatment of recurrent glioblastoma: preliminary observations in a patient with a favorable response to therapy. This special issue
2. Broder H, Anderson A, Kremen TJ, Odesa SK, Liau LM: MART-1 adenovirus-transduced dendritic cell immunization in a murine model of metastatic central nervous system tumor. This special issue
3. Dutta T, Spence A, Lampson LA: Robust ability of IFN-γ to upregulate class II MHC antigen expression in tumor-bearing rat brains. This special issue
4. Lampson LA: Beyond inflammation: site-directed immunotherapy. Immunol Today 19: 17–22, 1998
5. Lampson LA: Basic principles of CNS immunology. In: Winn HR (ed.) Youman's Neurological Surgery, 5th edn. WB Saunders, Philadelphia, 2004, in press
6. Lampson LA: Immune regulation in the brain: lessons from autoimmunity, implications for brain tumor therapy. In: Ali-Osman F (ed.) Human Brain Tumors. Humana, 2004, in press
7. Harling-Berg CJ, Park TJ, Knopf PM: Role of the cervical lymphatics in the Th2-type hierarchy of CNS immune regulation. J Neuroimmunol 101: 111–127, 1999
8. Prins RM, Graf MR, Merchant RE, Black KL, Wheeler CRJ: Thymic function and output of recent thymic emigrant T cells during intracranial glioma progression. This special issue
9. Yu JS, Lee PK, Ehtesham M, Samoto K, Black KL, Wheeler CJ: Intratumoral T cell subset ratios and Fas ligand expression on brain tumor endothelium. This special issue
10. Armstrong WS, Lampson LA: Cell-mediated responses in the nervous system: CTL vs. NK activity; role of MHC modulation. In: Keane RW, Hickey WF (eds) Immunology of the Nervous System. Oxford, 1997, pp 493–547
11. Oldstone MB, Blount P, Southern PJ, Lampert PW: Cytoimmunotherapy for persistent virus infection reveals a unique clearance pattern from the central nervous system. Nature 321: 239–243, 1986
12. Sedgwick J, Brostoff S, Mason D: Experimental allergic encephalomyelitis in the absence of a classical delayed-type hypersensitivity reaction. Severe paralytic disease correlates with the presence of interleukin 2 receptor-positive cells infiltrating the central nervous system. J Exp Med 165: 1058–1075, 1987

13. Withan TF, Villa L, Yang T, Pollack IF, Okada H, Robbins PD, Chambers WH: Expression of a soluble TGF-β receptor reduces tumorigenicity by regulating natural killer activity against 9L gliosarcoma *in vivo*. This special issue
14. Katoh M, Wilmotte R, Belkouch M-C, de Tribolet N, Pizzolato G, Dietrich P-Y: Survivin in brain tumors: an attractive target for immunotherapy. This special issue
15. Parsa AT, Miller JI, Eggers A, Odgen III AT, Anderson RC, Bruce JN: Autologous adjuvant linked fibroblasta induce anti-glioma immunity: implications for development of a glima vaccine. This special issue
16. Paul DB, Read SN, Kulprathipanja N, Gomez GG, Kleinschmidt-DeMasters BK, Schiltz PM, Kruse CA: γInterferon-transduced 9L gliosarcoma. Cytokine gene therapy and its relevance to cellular therapy with alloreactive cytotoxic T lymphocytes. This special issue
17. Rustamzadeh E, Low WC, Vallera DA, Hall WA: Immunotoxin therapy for CNS tumor. This special issue
18. Mintz A, Gibo DM, Madhankumar AB, Debinski W: Molecular targeting with recombinant cytotoxins of interleukin-13 receptor alpha-2-expressing glioma. This special issue
19. Lampson LA, Lampson MA, Dunne AD: Exploiting the lacZ reporter gene for quantitative analysis of disseminated tumor growth within the brain: use of the lacZ gene product as a tumor antigen, for evaluation of antigenic modulation, and to facilitate image analysis of tumor growth *in situ*. Cancer Res 53: 176–182, 1993
20. Lampson LA: Interpreting MHC class I expression and class I/class II reciprocity in the CNS: reconciling divergent findings. Microsc Res Tech 32: 267–285, 1995
21. Vanderlugt CL, Begolka WS, Neville KL, Katz-Levy Y, Howard LM, Eagar TN, Bluestone JA, Miller SD: The functional significance of epitope spreading and its regulation by co-stimulatory molecules. Immunol Rev 164: 63–72, 1998
22. Glick RP, Lichtor T, Panchal R, Mahendra A, Cohen EP: Treatment with allogeneic interleukin-2 secreting fibroblasts protects against the development of malignant brain tumors. This special issue
23. Ren H, Boulikas T, Söling A, Warnke PC, Rainov NG: Immunogene therapy of recurrent glioblastoma multiforme with a liposomally encapsulated replication incompetent Semliki Forest Virus vector carrying the human interleukin-12 gene – a Phase I/II clinical protocol. This special issue
24. Lesniak MS, Tyler BM, Pardoll DM, Brem H: Gene therapy for experimental brain tumors using a xenogenic cell line engineered to secrete hIL-2. This special issue
25. Meyer-Puttlitz B, Hayashi Y, Waha A, Rollbrocker B, Bostrom J, Wiestler OD, Louis DN, Reifenberger G, von Deimling A: Molecular genetic analysis of gianT cell glioblastomas. Am J Pathol 151: 853–857, 1997
26. Drummond KJ, Ashley DM, Lampson LA: Enhanced expression of transforming growth factor-β1 increases invasiveness in a syngeneic murine model of malignant astrocytoma. Cancer Res, 2003 (in press)
27. Fecci PE, Mitchell DA, Archer GE, Morse MA, Lyerly HK, Bigner DD, Sampson JH: The history, evolution, and clinical use of dendritic cell-based immunization strategies in the therapy of brain tumors. This special issue
28. Sloan AE, Parajuli P: Human autologous dendritic cell-glioma fusions: feasibility and capacity to stimulate T cells with proliferative and cytolytic activity. This special issue
29. Kondo Y, Lampson LA: Enhancing T cell surveillance of brain micro-tumor: different drugs optimize T cell entry to grey or white matter. Cancer Res 41: 192, 2000 (abstract #1226)
30. Lampson LA, Lampson MA, Dunne A, Sethna MP: Tumor/cytokine interactions within the brain; interactions between gamma interferon and visualizable brain tumor cells. Neurol 41(Suppl 1): 320, 1991
31. Wen PY, Lampson MA, Lampson LA: Effects of gamma-interferon on major histocompatibility complex antigen expression and lymphocytic infiltration in the 9L gliosarcoma brain tumor model: implications for strategies of immunotherapy. J Neuroimmunol 36: 57–68, 1992
32. Lampson LA, Hickey WF: Monoclonal antibody analysis of MHC expression in human brain biopsies: tissue ranging from 'histologically normal' to that showing different levels of glial tumor involvement. J Immunol 136: 4054–4062, 1986
33. Lampson LA: Cell-mediated immunotherapy directed against disseminated tumor in the brain. In: McL Black P, Schoene WC, Lampson LA (eds) Astrocytomas: Diagnosis, Treatment and Biology. Blackwell Scientific, 1993, pp 261–289
34. McCluskey LP, Lampson LA: Local immune regulation in the central nervous system by substance P vs. glutamate. J Neuroimmunol 116: 136–146, 2001
35. Lampson LA: New animal models to probe brain tumor biology, therapy, and immunotherapy: advantages and remaining concerns. J Neuro-Oncol 53: 275–287, 2001

Address for offprints: Lois A. Lampson, 221 Longwood Avenue/LMRC 111, Boston, MA 02115, USA; Tel.: 617-278-0363; Fax: 617-547-9580; E-mail: lampson@rics.bwh.harvard.edu; http://home.attbi.com/~lampson/